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INFLUENCE OF HYDROSTATIC PRESSURE ON THE DENATURATION OF STAPHYLOCOCCUS ANTITOXIN AT 65°C.

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The quantitative effects of increased hydrostatic pressure on reaction rates provide a measure of the direction and magnitude of the volume change of the reactants, $\Delta V\ddagger$, in going from the normal to the activated state.[1, 2] With large molecules, such as those of proteins, these volume changes may be relatively large. Kinetic analysis of the rate of destruction of the bacterial luminescent system at various pressures and at temperatures about 15° above the normal optimum of the luminescent oxidation has shown that a large increase in volume, presumably of the protein catalyst and amounting to approximately 71 ml. per gram molecule, takes place.[3] In the denaturation of human serum globulin and egg albumin solutions at 65°C. a very considerable volume increase of activation also takes place, as evidenced by the decrease in the rate of precipitation at pressures of 10,000 lb. per square inch in comparison with the rate at normal pressure.[4]

In addition to these changes in molecular volume during the processes of activation, large volume increases in going from the initial to the final state of reversible denaturation equilibria evidently occur in the bacterial luminescent system at above optimum temperatures, or at lower temperatures in the presence of alcohol, urethane, ether, chloroform and certain other substances which bring about protein denaturation.[5, 6, 7] Furthermore, it has been shown by dilatometric studies that the denaturation of several proteins is accompanied by an increase in volume.[8]

The specific rate of inactivation of tetanus antitoxin at 60 to 66°C. is not constant, but decreases during the course of the reaction, showing that the mechanism of the inactivation is more complex than a single first order rate process.[10] The denaturation of diphtheria antitoxin by urea shows a

similar deviation from first order kinetics, although the specific rate is independent of the initial concentration of antibody.[11, 12] In the present investigation we have measured the rate of decrease in antibody activity of an equine antitoxin for staphylococcus hemolysin at 65°C. under normal and increased hydrostatic pressure with the hope of obtaining a more complete picture of the mechanism of the inactivation.

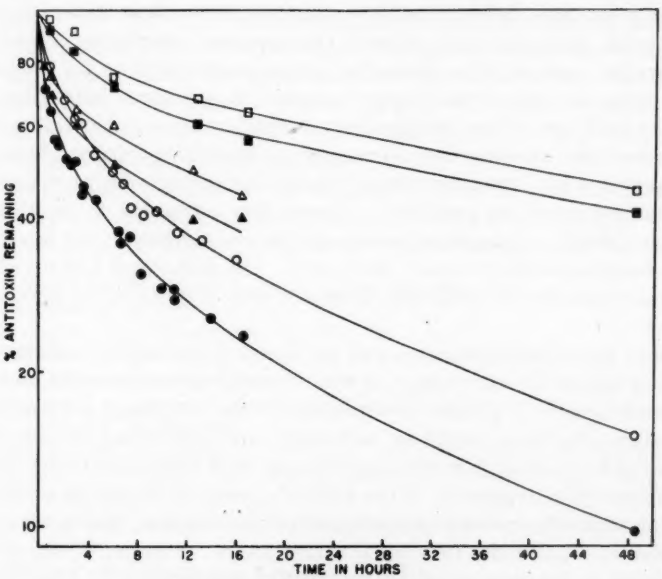
Methods.—The hemolysin solution was obtained from a semi-solid agar culture of *Staphylococcus aureus* in the usual manner.[9] The antitoxic globulin was obtained by fractionation of equine staphylococcus antitoxic plasma between 1.33 and 1.68 molar ammonium sulfate. The antiserum was supplied by The Lederle Laboratories, Pearl River, New York. The final stock globulin solution contained approximately 9 per cent globulin and 260 international units of antitoxin per milliliter.

Antibody solutions for denaturation were prepared by diluting the stock solution 1:62 in a buffer solution to give a protein concentration of 0.15 per cent. The pH 8.5 buffer was a boric acid, sodium borate and sodium chloride solution of ionic strength 0.1, the pH 4.82 buffer an acetic acid, sodium acetate and sodium chloride solution also of ionic strength 0.1, and the pH 6.65 buffer a $M/10$ sodium phosphate solution. Portions of each buffered solution of antibody were heated at normal pressure in a large, well-stirred water bath at 65 ± 0.015°C. in Wassermann tubes. For denaturation under pressure the tubes were cut in length to hold about 3 ml., stoppered by means of rubber stoppers leaving no air space, and placed in a water-filled steel pressure chamber. The chamber was connected to a hydraulic pump, pressure was applied and the entire chamber was placed in the water bath. The time required for the samples to come essentially to bath temperature was found to be two and one-half minutes outside the pressure chamber and five and one-half inside; correction was made in the time of denaturation for this lag in heating. The pH of the solutions did not change appreciably during the course of the heating.

The antitoxic activity of each partially inactivated antibody sample was assayed by making a series of dilutions of the antibody in intervals of 6 per cent, after which a constant amount of the toxin, containing about 150 hemolytic units, was added to each tube. The tubes were mixed and allowed to stand for a few minutes, after which washed rabbit erythrocytes were added as indicator. Dilutions were made in saline, buffered with sodium phosphate to pH 7.4. Hemolysis of half of the cells after incubation was taken as the end-point; since this was fairly sharp, the end-point dilution could be estimated by visual observation to within 3 per cent or less. In each group of tests a series of dilutions of unheated buffered antibody solution was included to serve as reference standard, and the per cent activity of the partially inactivated samples was computed as the ratio of the end-point dilution of the sample to the end-point dilution of

the unheated material. Several of the assays were repeated and the results averaged; agreement was good in practically all cases.

Results.—In figure 1 the per cent antitoxic activity of samples heated at 65°C. under 10,000 lb. pressure is plotted for various times of heating up to 48 hours. The results are also given for control experiments carried out under the same conditions except that the samples were held at atmospheric pressure. The combined results of a number of experiments at each pH



(FIGURE 1)

The per cent antitoxic activity remaining as a function of the time of heating at 65°C. under various conditions of pH and hydrostatic pressure, as follows: squares, pH 6.65; triangles, pH 4.82; circles, pH 8 .50. The solid marks refer to experiments at atmospheric pressure, the open marks to experiments at a pressure of 10,000 lb. per square inch.

are represented by a single curve. Each curve shows a rapid initial fall, followed by a slower decrease in activity, in this respect resembling the denaturation of tetanus antitoxin at 65°C.[10] and diphtheria antitoxin by urea at room temperature.[11, 12] At 10,000 lb. per square inch the curve has the same general shape as at normal pressure, but the rate, especially in the initial period of rapid denaturation, is considerably less. The results obtained in a few experiments at intermediate pressures fell between the data for atmospheric pressure and the data for 10,000 lb. At lower pH

values in phosphate and in acetate buffer (Fig. 1) the antibody is more stable, and the pressure effects appear to be less marked.

It was demonstrated that the products of denaturation do not affect the titre, as shown by control experiments in which mixtures of largely denatured antibody and untreated antibody were assayed. Thus the apparent decrease in specific reaction rate is not due to such a possible error in titration. For samples heated at pH 8.50 for 150 minutes the per cent denatured is independent of the initial antibody concentration over a range from three times as much to one-third as much as that ordinarily employed, indicating that, despite the apparent complexity of the denaturation reaction, it is a first order process with respect to the antibody. The same amount of inactivation occurred in specimens heated for the same total length of time whether heated continuously or for several periods between which the specimens were cooled to room temperature. Although inspection of the data demonstrates clearly that pressure decreases the rate of inactivation of the antibody, a quantitative expression of this effect is rather difficult to formulate, because of the complexities of the denaturation reaction and the pressure effect on it. The data at pH 8.50 appear to warrant quantitative treatment, however, such as given in the next paragraph.

Since the effect of pressure was not constant during the reaction but greater during the early stages, it was impossible to evaluate the effect of pressure simply by graphic transposition of the data along a logarithmic time plot; the curves could not be brought into coincidence by this treatment. The decrease in specific reaction rate with time could be accounted for either by heterogeneity of the antibody groups or by simultaneous reactions with different rate constants and volume changes. The latter might involve an equilibrium between a native and a "protected" form of the antibody, as has been postulated for the urea denaturation.[12] This mechanism requires that the activity equal the sum of two exponential expressions. This requirement was satisfied by the present data within the experimental error, but since the data are not extensive enough for a really satisfactory test of adherence to this expression, the volume changes have been calculated simply by comparison of the slopes of the two denaturation curves at equal activities of antibody. By this method the apparent volume change of activation was found to be 39 ml. per mole between 100 and 70% activity, 24 ml. per mole in the neighborhood of 40% and 16 ml. per mole at 20%. Interpretation of the change in $\Delta V\ddagger$ is difficult at present; it is possible that the over-all reaction may involve at the start a larger portion of each molecule than later on.

In direction and magnitude the effects of pressure are similar in antitoxin inactivation and in certain protein denaturations.[3-7] Additional evidence is thus provided of significant features common to antibody and

protein denaturations.[11, 12] Further studies on the influence of pressure in relation to temperature, pH and denaturants should considerably aid in understanding the mechanism of the reactions.

Summary.—At 65°C. denaturation of antitoxin against staphylococcus hemolysin occurs rapidly during the initial part of the reaction. The specific rate decreases as the reaction proceeds, but appears to be independent of the initial concentration of antibody. Hydrostatic pressure (up to 10,000 lb. per square inch) retards the velocity of denaturation; this fact shows that there is a volume increase of the molecules in going from the normal to the activated state. Pressure exerts a greater influence on the reaction during the initial stages of the reaction than during the later stages, and the apparent volume increase of activation changes from 39 ml. per mole during the first 30% of inactivation to 16 ml. per mole after 80% of the antibody has been inactivated.

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A COMPARISON BETWEEN THE POSITION EFFECTS OF NORMAL AND MUTANT ALLELES

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It has been reported recently[1] that the effect of the recessive mutant allele *cubitus interruptus* (*ci*), located in the small fourth chromosome of *Drosophila melanogaster*, is frequently changed when a rearrangement involving the fourth chromosome has been produced. While the heterozygote between the mutant *ci* and a normal allele + is nearly always normal when no rearrangement is present, many of the heterozygotes between a "position allele,"[2] *R*(*ci*), and a normal allele show interruptions of the cubital veins. A comparison of the degree of interruption in the heterozygotes *R*(*ci*)/+ with that reported by Dubinin and Sidorov as the position effect of a normal allele of *ci* in rearranged fourth chromosomes of flies with the constitution $R(+)/ci^{2}$·[4] showed that the latter constitution seemed to produce more extreme interruptions than the former. Since, however, the strength of the position effects varies with different rearrangements both for *R*(+) and *R*(*ci*) position alleles, and since the genetic background of the *R*(+) stocks used by the Russian investigators was presumably different from that present in our *R*(*ci*) stocks, a study of the *R*(+) and *R*(*ci*) effect on comparable material was indicated. This paper reports the results of such a comparison.

Heterozygotes of Position Alleles R(ci) and a Normal Allele: R(ci)/+.— Fifty-five translocations between a fourth chromosome carrying *ci* and one or more of the chromosomes Y, 2 or 3 were discovered, in the offspring of irradiated males which had been made isogenic except for chromosome 4 with an isogenic wild-type stock, Canton-S. Three of these translocations had been lost before the present investigation was started. The remaining fifty-two were tested for the presence of a position effect in heterozygotes with a normal allele of *ci* called $+^{2}$, present in a stock homozygous for the eyeless-allele ey^{2}. This $+^{2}ey^{2}$ stock likewise had been made isogenic with the Canton-S stock, except for chromosome 4. Since it had been found that at 26°C. not only nearly all heterozygotes *ci*/+ but also most *R*(*ci*)/+ flies are normal, and since it was known that the lower temperatures enhance the expression of the *ci* phenotype, the cultures were raised at approximately 17°C. Heterozygotes of constitution *ci*/+, which do not show the position effect, were reared as sibs of *R*(*ci*)/+, and served as controls. With few exceptions, at least three and sometimes more than ten cultures of the parental genotypes $+^{2}ey^{2}/+^{2}ey^{2}$ females by single

$R(ci)/ci\ ey^R$ male were raised for each position allele. Such crosses yielded round-eyed $R(ci)+^{ey}/+^2ey^2$, and small-eyed $ci\ ey^R/+^2ey^2$ offspring.[5] These were classified individually as to degree of interruption in the section between the posterior crossvein and the wing margin of the cubital vein. Originally, the same classification was used as reported in previous papers, in which normally uninterrupted veins were denoted as *N*, uninterrupted but partially thinned veins as 0, interruption of less than one-half of the vein section as 1, of more than one-half as 2, and complete, or nearly complete, absence of the whole section as 3. For purposes of comparison a mean for the degree of interruption of a group of flies was calculated after giving arbitrary values 0, 1, 2, 3 and 4 to the five phenotypic classes *N*, 0, 1, 2 and 3. This latter classification will herewith be abandoned and be replaced by the classification 0 (formerly *N*), 1 (formerly 0), 2 (formerly 1), 3 (formerly 2) and 4 (formerly 3). While the change in nomenclature may be conducive to some confusion, it is believed that it will simplify the presentation of data to be reported in this and future articles. Females and males will be treated separately because of different degrees of interruption of like genotypes in the two sexes.

The control females $ci/+^2$ were overwhelmingly (9520 individuals) normal, i.e., of Class 0, with 34 of Class 1 and no fly more extreme than Class 1. This gives a mean value of 0.0036 for expressivity. Of 10,235 control males, 10,097 were of Class 0, 134 of Class 1, and 4 of Class 2, giving a mean of 0.0139. Information on the mean values for each of the $R(ci)/+^2$ heterozygotes is summarized in lines 1 and 3 of table 1.

TABLE 1

EXPRESSIVITY OF 52 DIFFERENT $R(ci)$ ALLELES IN HETEROZYGOTES $R(ci)/+^2\ ey^2$ (17°C.) AND 19 DIFFERENT $R(+)$ ALLELES IN HETEROZYGOTES $R(+)/ci\ ey^R$ (26°C.). EACH ENTRY REPRESENTS THE NUMBER OF ALLELES GIVING A MEAN VALUE OF EXPRESSIVITY WITHIN THE RANGE INDICATED

	0.00–0.09	0.10–0.49	0.50–0.99	1.00–1.49	1.50–1.99	2.00–2.49	2.50–2.99	3.00–3.49	3.50–4.00
$R(ci)/+^2\ ey^2$ ♀♀*	25	9	6	3	2	4	2	..	..
$R(+)/ci\ ey^R$ ♀♀	..	..	..	5	5	6	1	2	..
$R(ci)/+^2\ ey^2$ ♂♂	23	6	8	4	5	4	2	..	..
$R(+)/ci\ ey^R$ ♂♂	..	..	1	1	8	4	3	1	1

* One $R(ci)$ allele could not be introduced into females.

The distributions of the different $R(ci)$ alleles should not be taken as final in detail. The errors attached to the individual values have not been calculated but would undoubtedly not exclude slight shifts in the distributions. Moreover, in a few cases the different cultures of a given $R(ci)$ allele were significantly different in mean values. The causes of such differences are not known except for the probability that an extra fourth chromosome, containing $ci\ ey^R$ was present in some parents. This would lead to a triplo-

IV condition in part of the offspring and result in a shift of venation types toward normality.[6] In general, each mean value for $R(ci)/+$ flies entered in table 1 is based on the total distribution of flies from all cultures. It is seen that the expression of the phenotypes of the fifty-two different $R(ci)/+$ groups varies, with two exceptions in each sex, between the mean value 0 (all wings normal) and less than 2.50 (wing interruption approximately one-half of relevant vein section). The four mean values which are more extreme are only slightly so; the two values for females both being 2.50, and the two values for males being 2.51 and 2.64, respectively.

Heterozygotes of Position Alleles $R(+)$ and the Allele ci: $R(+)/ci$.—In order to obtain $R(+)$ alleles males of a Canton-S stock were x-rayed. This stock was isogenic except for chromosome 4 with the *ci* stock from which the $R(ci)$ alleles were derived. The x-ray dosage was 4000 r. The irradiated males were mated to non-isogenic females of the constitution attached-*X y; ci ey*^{R}. The F_1 females and males raised at 26°C. were heterozygous for *ci* and were carefully inspected as to the appearance of the cubital vein. Among 1990 females, 17 individuals showed thinning, or interruption of the vein and among 2047 males, 12 such individuals were found. The distribution of these flies over the four abnormal venation classes

	1, 2, 3 and 4
for females was	1, 8, 5 and 3, respectively
and for the males	4, 5, 3 and 0, respectively.

Each of these 29 flies was mated individually to *ci ey*^{R} (isogenic stock). The offspring of the fertile cultures consisted of round-eyed and eyeless flies. Round-eyed males were selected and back-crossed for about ten generations to *ci ey*^{R} flies from stock, in order to approach isogeneity with the *ci ey*^{R} stock except for the irradiated fourth chromosome carrying the wild allele $+^C$ from Canton-S. This procedure was not possible for three of the original twenty-six cultures since the round-eyed males proved to be sterile. A consideration of these cultures derived from one female each of Classes 2, 3 and 4 will be omitted since isogeneity could not be attained. There remained thus twenty-three cultures, derived separately from flies with a *ci* phenotype. Four of these cultures, from three original females and one male, all of Class 2, in later generations failed to show *ci* phenotypes and linkage tests proved absence of any translocation. The four ancestors of these cultures either were rare phenotypic overlaps of typical heterozygotes $+^C/ci$ or possibly were gonosomic mosaics[7] in which an $R(+)$ allele had participated in the formation of the somatic tissues but no rearrangement had entered the nuclei which went to form the gonads.

The expressivity of $R(+)$ alleles in the heterozygote $R(+)/ci$ was studied in the nineteen different stocks finally available. No compre-

hensive chromosomal analyses of these stocks are yet at hand but on the basis of Khwostova's cytological analysis[4] of 196 changed + alleles, all but three of which proved to be rearrangements, it is assumed that the nineteen changed + alleles of the present study are $R(+)$ alleles. Mean values of expressivity were calculated for an average of five separate cultures of each $R(+)$ allele. In most cases the cultures of a given $R(+)$ allele gave similar values, but in some cases non-homogeneity was apparent. That this was probably due to presence of an extra fourth chromosome carrying *ci* ey^R, was confirmed genetically and cytologically in a number of individuals. Total mean values were determined from the sum of the distributions of all cultures of each $R(+)$ allele. These values, for the females, lie between 1.03 and 3.44, for the males between 0.96 and 3.80 (table 1, lines 2 and 4).

Comparison Between the Position Effects of Heterozygotes $R(ci)/+$ *and* $R(+)/ci$.—The data for this comparison are available in table 1. There is no doubt that all values of $R(+)/ci$ types represent striking position effects, since heterozygotes between *ci* and the $+^C$ allele in its typical position, at 26°C., are nearly always normal, i.e., give a mean of 0.00 in contrast to the lowest observed $R(+)/ci$ value of 0.96. It is more difficult to determine which of the $R(ci)/+$ values represent only low values of typical $ci/+$ heterozygotes raised at 17° and which $R(ci)/+$ values can be regarded as characterizing true position effects. For purposes of comparison with $R(+)/ci$ values, it will be conservative to regard only those $R(ci)$ alleles as giving position effects whose means in both sexes are larger than 0.10. This eliminates for purposes of comparison, all 25 $R(ci)$ alleles giving mean values of less than 0.09 for the females. Twenty-three of these give similarly low values for the males. The other two $R(ci)$ alleles have mean values, in males, in the range 0.10–0.89. They, together with a third in the same range, not represented among the female heterozygotes, have to be subtracted from the six means for males listed in the range 0.10–0.49. This reduces to twenty-six the number of $R(ci)$ alleles available for the comparison with $R(+)$. The two distributions, in both sexes, are significantly different, indicating a greater mean position effect for $R(+)$ alleles than for $R(ci)$ alleles.

Before accepting this conclusion it must be pointed out that the data from the two types of position alleles are not strictly comparable: (1) the normal allele of *ci* present in $R(+)$ is the Canton-S allele $+^C$ while that used in the heterozygotes with $R(ci)$ is the $+^2$ allele; (2) the culture temperature for $R(ci)/+$ was 17°C., that for $R(+)/ci$ 26°C. Difference (1), between $+^C$ and $+^2$, should act in the direction of making $R(+^C)/ci$ more extreme than $R(ci)/+^2$. However, this effect would be very small.[5] On the other hand, an overwhelming effect acting in the opposite direction on the mean values of $R(+)/ci$ vs. $R(ci)/+$ is due to difference (2). In-

deed, at 26°C. most $R(ci)$ alleles in the combination $R(ci)/+$ give nearly exclusively normal wings. Thus in experiments at 26°C. involving nine different $R(ci)$ alleles, the total mean for the females was 0.005 and for the males 0.009 with the maximum individual means being 0.057 and 0.043, respectively. At 17°C. the individual means for these same $R(ci)$ alleles lie between 0.300 and 2.083 for the females, and between 0.516 and 2.066 for the males. The low temperature actually used in the experiments with $R(ci)$, summarized in table 1, serves to separate phenotypically the position effect yielding $R(ci)$ alleles from the typical *ci* allele in its normal position and from those $R(ci)$ alleles which do not give position effects. Had the experiments with $R(ci)/+$ flies been conducted at the same high temperature as those with $R(+)/ci$ flies the difference in the two distributions would have been much more striking. There would have been very little overlap of the two distributions in either sex since all $R(ci)/+$ values would lie close to 0, that is, to the left of the $R(+)/ci$ values.

There is, however, another factor which must be considered. All $R(+)$ alleles found produced position effects whose mean values either approached a minimum value of 1 or were more extreme. Why were no lower values observed? It is obvious that this is at least partly due to the way in which the $R(+)$ alleles were discovered, namely, as single individuals showing a vein disturbance of the degree class 1, 2, 3 or 4. Had position alleles originated whose mean effect is very low, as, for instance, 0.1, then most individuals carrying such an allele would appear normal and the probability of the single original fly having abnormal venation and therefore being discovered would be small. Even for $R(+)$ alleles giving mean values of about 1 the probability of overlap with normal is considerable. These considerations make it apparent that the left end of the distribution of $R(+)/ci$ mean values is selectively depressed. It can, however, hardly be assumed that this effect is strong enough to obliterate the differences between the $R(+)/ci$ and $R(ci)/+$ distributions. As stated before, at a temperature of 26°C. most, if not all $R(ci)/+$ values would have shifted to the left of all present $R(+)/ci$ values so that only an improbable large number of undiscovered low $R(+)$ alleles could make the two distributions alike.

While this analysis upholds the statement that $R(+)$ alleles give a greater mean position effect than $R(ci)$ alleles, it was decided to add a direct test. Males homozygous for the constitutions *ci* ey^R and $+^C$ were irradiated simultaneously with a dose of 4000 r and then crossed to $+^C$ and *ci* ey^R females, respectively. Equal numbers of the two reciprocal crosses were made and the F_1 inspected for venation types. In order to exclude any bias the cultures were coded by a third person so that the investigator did not know to which cross any culture belonged before the counts were completed. As a control reciprocal crosses were made of the same type,

but with non-irradiated P males. In the control cross $+^C$ ♀♀ × *ci ey*^{R} ♂♂ among 1557 ♀♀ and 1855 ♂♂ only normal, Class 0 wings were encountered, and in the reciprocal control cross *ci ey*^{R} ♀♀ × $+^C$ ♂♂, among 1675 ♀♀ and 2037 ♂♂, only a single male with very weak Class 1 venation. The results of the irradiation experiments are strikingly different (table 2). While the great majority of F_1 flies still showed normal

TABLE 2

DISTRIBUTION OF PHENOTYPES OF F_1 INDIVIDUALS FROM CROSSES OF (*a*) $+^C$ ♀♀ × IRRADIATED *ci ey*^{R} ♂♂ AND (*b*) *ci ey*^{R} ♀♀ × IRRADIATED $+^C$ ♂♂. 4000 r. 26°C.

	P	F_1	0	1	2	3	4
(*a*)	$+^C$ ♀♀ × *ci ey*^{R} ♂♂	♀♀	2328	3	..	..	..
		♂♂	2311	2	..	..	..
(*b*)	*ci ey*^{R} ♀♀ × $+^C$ ♂♂	♀♀	2219	5	9	10	1
		♂♂	2139	2	13	6	..

venation, five of the F_1 individuals from the irradiated *ci ey*^{R} males and forty-six from irradiated $+^C$ males had *ci* type venation. These fifty-one individuals represent, with the possible exception of a very few weak *ci* types, position alleles. In spite of equal probability in this experiment of discovering $R(ci)$ and $R(+)$ alleles the inequality of the actual finding, namely, 5:46, is proof of an intrinsic difference. Moreover, all five $R(ci)$ alleles appeared as members of the least extreme Class 1 while only seven of the forty-six $R(+)$ alleles belong to Class 1, the rest being nearly equally distributed over Classes 2 and 3 except for one belonging to Class 4. The experiment bears out the original contention of greater intensity of position effects produced by $R(+)$ as compared to $R(ci)$ alleles.

Still additional support for this result comes from a comparison of the differences between the relevant $R(ci)/+$ mean values reported in table 1 with the mean values for *ci/ci* flies raised at similar temperatures, as calculated from earlier data[9] and the differences between the $R(+)/ci$ values reported in table 1 and the mean values of their *ci/ci* sibs. None of the fifty-two values of both sexes for the heterozygotes between $R(ci)$ and + is larger, i.e., more extreme than the values for homozygous *ci/ci*, while ten of the thirty-eight heterozygotes between $R(+)$ and *ci* give values larger than the corresponding *ci/ci* means. This peculiar phenomenon will be discussed in another communication. It may be concluded that in the average, the position effects of $R(+)$ alleles are stronger than those of $R(ci)$ alleles.

Discussion.—The main result reported in this paper is the fact that in cases of rearrangements the effect of the heterozygote $+/ci$ differs according to whether the break in the fourth chromosome is present in the homologue carrying the allele +, or the allele *ci*. In the first case, the heterozygote produces a more extreme mean interruption than in the second. In view of the demonstration[6] that both the normal alleles and the

mutant allele *ci* act toward uninterrupted venation, but with different effectiveness, a more extreme interruption indicates a decreased effect in terms of primary genic action. If it is assumed that the new position of a gene involved in a position allele is responsible either for a change in its functioning or in a decrease in amount of available substrate for its activity, then the result of comparison of $R(+)/ci$ with $R(ci)/+$ conforms to a reasonable expectation. A rearrangement might well produce a greater impact on the heterozygote $+/ci$ if a break is close to the + allele which in a normal position carries the burden of leading to more or less normal phenotypes than if the break is close to the mutant *ci* allele whose effect is small in comparison to that of +.

Ephrussi and Sutton[10] have recently proposed a special interpretation "which regards position effect as a result of chromosome pairing which causes a modification of stress near to the affected loci." This interpretation, related to an earlier one by Muller,[11] would lead to the expectation of identity in effect of $R(+)/ci$ and $R(ci)/+$ since a distortion by pairing forces from one homologous chromosome to the other should act symmetrically on the two alleles regardless whether they lie in the unchanged or rearranged chromosome. Without additional assumptions the hypothesis of pairing stresses is not supported by the finding of different mean effect of $R(+)/ci$ and of $R(ci)/+$.

Summary.—In *Drosophila melanogaster* heterozygotes between the mutant gene *ci* (*cubitus interruptus*) and a normal allele $+^2$ or $+^C$ are mostly normal in phenotype. If a rearrangement has taken place near to the *ci* locus the heterozygotes show varying degrees of vein interruptions. This position effect is of different strength according to whether the rearrangement involves the chromosome with a normal allele, $R(+)$, or its homologue with the mutant allele, $R(ci)$. While different position alleles of either type lead to different mean values for degree of expression, the distribution of means for twenty-six types of $R(ci)/+$ flies is significantly less extreme than that for nineteen types of $R(+)/ci$ flies. The significance of these results on interpretations of position effects is indicated.

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[1] Stern, C., and Heidenthal, G., these PROCEEDINGS, **30**, 197–205 (1944).

[2] The original definition of the term "position allele" as given in the paper cited in footnote 1 is somewhat extended in this paper. Instead of using "the symbol $R(ci)$ for any rearrangement (R) which leads to a position effect of *ci*" we shall rather use the symbols $R(ci)$ and $R(+)$ for any rearrangement involving the neighborhood of the *ci* locus regardless as to whether or not a position effect results from such a rearrangement. This extension becomes necessary on account of the practical difficulty of deciding, in

borderline cases, whether or not a changed effect results from a rearrangement (see below in text).

[3] Dubinin, N. P., and Sidorov, B. N., *Biol. Zhurnal*, **3**, 307–331 (1934).

[4] Khwostova, W. W., *Bull. Acad. Sci. U. S. S. R. Serie biologique*, pp. 571–574 (1939).

[5] Except in the table and in cases of special emphasis, references to the *ey* alleles will be omitted in the following.

[6] Stern, C., *Genetics*, **28**, 441–475 (1943).

[7] Sidky, A. R., *Jour. Genetics*, **39**, 265–272 (1940).

[8] Stern, C., and Schaeffer, E. W., these PROCEEDINGS **29**, 361–367 (1943).

[9] No data are available for *ci/ci* flies raised at 16°C. Data collected at 18°C. (see paper quoted under reference 6, tables 12 and 13), which should give less extreme means than 16°C. cultures, provide means of 2.68 and 3.11 in two different experiments for females and 3.07 for males. All these figures are larger than those for any $R(ci)/+$ value.

[10] Ephrussi, B., and Sutton, E., these PROCEEDINGS, **30**, 183–197 (1944).

[11] Muller, H. J., *Summ. Commun. XV int. physiol. Congr.* (*Leningrad-Mosc.*) pp, 286–289 (1935); and *Proc. 15th Int. Physiol. Congr.* (*Leningrad-Mosc.*), pp. 587–589 (1938).

LINKAGE IN THE ALBINO CHROMOSOME OF THE RAT

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The first known case of linkage in the rat was discovered by Castle and Wright in 1916.[1] Repulsion in F_1 hybrids between red-eyed yellow (r) and pink-eyed yellow (p) was demonstrated. Later it was found that both these characters show linkage with albinism (c). The common chromosome pair in which these three genes are borne has been designated the albino or first (I) chromosome pair.

Intensive study was subsequently made of the interrelations of these three genes by Castle, Dunn[2] and Wachter.[3] They found that crossing-over occurs more freely in female than in male F_1 hybrids. In the former the cross-over percentage between c and p was found to be 21.93 ± 0.44; between p and r, 20.46 ± 0.92; and between c and r, 0.53 ± 0.78. The values found for males were comparable but smaller, viz., 18.4 ± 0.3, 15.5 ± 1.0 and 0.18 ± 0.50, respectively. Consequently the order of the three genes was unmistakably $c\ r\ p$.

A gene for waltzing (w) discovered by Dr. Helen Dean King in 1936[4] was shown by King and Castle[5] in 1937 to be linked with albinism with about 45 per cent of crossing-over The exact figures were 44.7 ± 0.7 based on the raw data, 45.8 ± 0.7 based on the data corrected for lack of penetrance of waltzing. Since the gene for waltzing (w) was obviously more distant

from *c* than either *r* or *p*, it was assumed that the order of the genes was *c r p w*.

To test this assumption further and in particular to ascertain whether linkage would (as expected) be shown to exist between *p* and *w*, a three-point cross was made involving the three genes *c*, *p* and *w*.

The results of this cross were reported by Castle and King in 1941[6] (correction being made for low penetrance of waltzing) as showing, (1) a percentage of crossovers between *c* and *w* of 42.6, which is in fair agreement with the earlier reported 45 ±; (2) a percentage of crossovers between *c* and *p* of 22.9 ± 1.2, which is in fair agreement with the earlier finding of Castle and Wachter, of 21.93 ± 0.44; (3) a percentage of crossovers between *p* and *w* of 35.3. It was concluded that chromosome I of the rat contains five recessive genes in the order *l c r p w*, *l* standing for the lethal shown by Grüneberg to lie to the left of *c* at a distance of 3.3 units.

But Whittinghill (1944)[7] upon subsequent examination of the data challenged our conclusions both as to the existence of linkage between *c* and *w*, and as to the existence of linkage between *p* and *w*. He showed that while we had made allowance in our report on the three-point cross for lack of penetrance of *w*, we had failed to make allowance for double crossing-over. His argument was based on the gene sequence which we had assumed, *c p w*.

In view of Whittinghill's criticism, a recalculation (Castle, 1944)[8] was made of the data from the three-point cross, allowance being made for double crossing-over as well as for lack of penetrance of *w*. The conclusion was reached that the crossover percentage between *p* and *w* was 32.2 on the uncorrected data, *but 45.4 on the corrected data*. If this latter value were accepted as correct, it would indicate that *w* was even more remote from *p* than it was from *c*, the distance *p w* being 45.4, whereas *c w* was on the evidence of the three-point cross 40.6 ± 1.4 and on the evidence of earlier and more extensive experiments about 45.

My colleague, Dr. E. R. Dempster, having read the discussion, suggested that *c* might be located *between* *p* and *w*, contrary to the order assumed in our discussion to be *c p w*. The same suggestion was made independently by Professor L. L. Burlingame of Stanford University, who has worked out the statistical problem in detail. I am deeply grateful for his kindness in giving me the benefit of his study of the data.

If we assume the order of the genes in the three-point cross to be *p c w*, as suggested by Drs. Dempster and Burlingame, the constitution of the F_1 animals employed in the test backcross was $\frac{p\ C\ W}{P\ c\ w}$, their gametes would be, as indicated in table 1, of eight possible sorts. The numbers of young resulting in each back-cross class are shown for simplicity only in their

corrected form, i.e., with allowance made for low penetrance of waltzing. The raw data are given in the earlier papers of 1941 and 1944.

From table 1 it will be seen that the crossing-over in region 1 (between *p* and *c*) indicated by the colored classes is 35.8 + 24.2 = 60.0, which is 21.3 ± 2.0% of the colored population, 282. This value 21.3 agrees well with the value for crossing-over between *p* and *c* obtained by Castle and Wachter, viz., 21.9 ± 0.4.

For region 2 (between *c* and *w*) the indicated crossovers are 92.4 + 24.2 = 116.6, which is 41.3 ± 2.0% of the colored population. This agrees fairly well with the earlier estimates of crossing-over between *c* and *w*, as being about 45%. It establishes beyond question the existence of linkage between albinism and waltzing.

TABLE 1

RESULTS OF THE TEST CROSS $F_1 \frac{p\ C\ W}{P\ c\ w} \times p\ c\ w$

GAMETES OF F_1	CROSSOVERS IN REGION	GRAY	YELLOW	ALBINO
p C W	0	...	129.6 non-waltzers	...
P c w	0	...	...	(145.2) waltzers
P C W	1	35.8 non-waltzers	...	...
p c w	1	...	...	(145.2) waltzers
p C w	2	...	92.4 waltzers	...
P c W	2	...	...	(95.8) non-waltzers
P C w	1 and 2	24.2 waltzers	...	...
p c W	1 and 2	...	...	(95.8) non-waltzers
Totals		60.0	222.0	241.0

The total amount of crossing-over between *p* and *w* indicated by the colored classes in table 1, if the *c* locus is left out of consideration, would be 45.4 ± 2.0%. This is a fairly significant indication of linkage between pink-eyed yellow (*p*) and waltzing (*w*), which was a prime objective of the investigation.

If now we accept the conclusion that *c* lies between *p* and *w*, it becomes necessary to reconsider the position of two other genes, *r* and *l* in the chromosome I map. It is known that *r* lies between *p* and *c*, very close to the latter. Grüneberg has reported that *l* (a lethal) lies at 3.3 units from *c* on the side away from *p*. This would place it between *c* and *w*. Consequently the map will become on the best evidence at present available (based primarily on crossing-over in females):

$$\frac{p\ 20.5\ r\ 0.5\ c\ 3.3\ l \qquad 42? \qquad w}{0 \qquad\qquad\qquad\qquad\qquad\qquad 66.3}$$

[1] Castle, W. E., and Wright, S., *Carnegie Inst. Wash. Publ.*, **241**, 175 (1916); **288**, 29 (1919).
[2] Dunn, L. C., *Genetics*, **5**, 325 (1920).
[3] Castle, W. E., and Wachter, W. L., *Genetics*, **9**, 1 (1924).
[4] King, H. D., *Jour. Mam.*, **17**, 157 (1936).
[5] King, H. D., and Castle, W. E., these PROCEEDINGS, **23**, 56 (1937).
[6] Castle, W. E., and King, H. D., *Ibid.*, **27**, 394 (1941).
[7] Whittinghill, M., *Ibid.*, **30**, 221 (1944).
[8] Castle, W. E., *Ibid.*, **30**, 226 (1944).

INDUCED MUTATIONS AND POSSIBLE MECHANISMS OF THE TRANSMISSION OF HEREDITY IN ESCHERICHIA COLI

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One of the most remarkable facts brought to light by genetics is the fundamental similarity in the most diverse organisms of the basic mechanisms of the transmission of heredity from parents to offspring. The principal carriers of heredity appear generally to be discrete corpuscles, genes. Only in bacteria and in other asexual lower organisms is the nature of the hereditary transmission still unclear. The most efficient method in use for the detection of genes—namely, the crossing of different strains and observation of segregations in the offspring of the hybrids—is not applicable to asexual forms. The problem must be approached, then, with the aid of the less direct, yet in the final analysis reliable, method of studying mutations, particularly those induced by irradiation. As materials for investigations on the induction of mutations by radiation, bacteria have, in fact, certain advantages not found in higher organisms.

Notwithstanding the large amount of work that has been done in radiation genetics, the events that lead to the manifestation of changes in genes in irradiated cells are not well understood. One important reason for this situation is that in the materials most studied (Drosophila, maize) the effects of irradiation cannot be determined until after a considerable number of cell divisions have gone by following the treatment. Since in bacteria it is possible to observe induced changes soon after they occur, the work to be described here was undertaken in the hope that the results might contribute toward a better understanding of the origin of these changes.

It is known from the work of Luria and Delbrück[1] and of Demerec and Fano[2] that in the *B* strain of *Escherichia coli* spontaneous mutations to resistance to the bacteriophage now known as T1 occur with a frequency of about 1×10^{-8} per generation, and that at least two types of such mutants can be recognized. The more common type forms large colonies,

while the other forms tiny colonies. In this study the over-all mutability of *B* to resistance to T1 (*B/1*) was investigated without separating the two types of mutants.

Materials.—The mutant strain known as *B/r* (*B* resistant to radiations), which came from the *B* strain of *E. coli*, was used in these experiments. This mutant, found and studied by Mrs. Witkin,[3] was selected for our work because it is more resistant than the original *B* strain to both ultraviolet and x-ray irradiation. Since it can stand treatment with higher dosages of both radiations than the *B* strain, it was hoped that increases in the mutation rate would be more easily detectable by irradiating *B/r*. This strain was used in all experiments mentioned in this paper. For reasons of convenience, the abbreviated symbol, *B*, instead of the full symbol *B/r* will be used here.

Bacteriophage T1, known also as alpha and as P28, was used to isolate mutants resistant to that phage.

Methods.—The standard method used in this laboratory for determining how many bacteria resistant to a certain phage are present in a population of sensitive bacteria is, first, to plate on nutrient agar in a Petri dish about 5×10^8 phage particles and, a few minutes later, to plate on the same dish the bacteria to be tested. When this procedure is followed the phage comes into contact with the bacteria and lyses all those that are sensitive to the particular phage used, while the resistant ones survive to form colonies. By counting these colonies, the number of resistant bacteria among the total number plated is determined.

In our experiments it proved necessary to detect not only the number of *B/1* mutants (bacteria resistant to T1 phage) that showed up before the bacteria had had a chance to divide, but also the number that appeared after the bacteria had passed through a certain number of divisions. It was not satisfactory to let the bacteria divide in broth and then determine the number of *B/1* individuals by the standard method, because after several divisions it would have been impossible to distinguish between *B/1* bacteria that had originated as mutations and those that had resulted from division of *B/1* mutants present in the culture as a result of mutations occurring during one of the earlier divisions.

An effective method was developed, which satisfies the requirements of our experiments. A known number of bacteria is plated on nutrient agar medium on Petri dishes. These are incubated for the period of time necessary for the bacteria to pass through the desired number of divisions, and then the phage is sprayed over the surface of the plates as a fine aerosol. For this purpose we use a De Vilbiss glass nebulizer number 44. A stock suspension, containing about 5×10^9 phage particles per milliliter, is placed in the chamber of the nebulizer and the aerosol is generated by an air flow of six liters per minute, regulated by a Linde Oxygen Therapy

Station Flow Meter. Each plate is exposed to the phage aerosol for 1 minute by holding it close to the mouth of the nebulizer and moving it around during the exposure so that all regions of the plate are uniformly covered. Tests have shown that when this procedure is followed a sufficient number of phage particles adhere to the plate to produce complete lysis of sensitive bacteria, while resistant bacteria continue to divide and form colonies. The main advantage of this method is that the positions of the bacteria in a growing colony are not disturbed by application of the phage, and that therefore all mutations occurring within a certain period are represented by single colonies, no matter how many mutant bacteria have been formed by division of the original mutant individual. During the development of this method, tests were made of 201 colonies that appeared on a number of plates after exposure to T1 aerosol. All were found to be resistant to T1 and sensitive to another phage active on *B* (T2), indicating that the exposure to the phage had been sufficient to eliminate all sensitive bacteria without introducing any contaminant.

When this aerosol method for applying phage is used, it is important to take precautions against contaminating the laboratory with the phage. If spraying is done in the open the aerosol spreads rapidly from room to room and the phage may persist for several days. To prevent this, spraying is done in a box, measuring 25 × 25 × 23 cm., with a glass top, an opening on one end for the nebulizer, an opening on the other end with a cloth sleeve through which the arm holding a Petri dish is inserted, and an opening on one of the two sides with a tube through which air is sucked out of the box. During the operation, air containing the phage aerosol is drawn out from the box at a slightly higher rate than it is blown in through the nebulizer, and is passed through a heated copper coil to destroy the phage.

For ultraviolet treatment we used a General Electric germicidal lamp, about 80 per cent of whose output is radiation of wave length 2537 A. In all the experiments, the material was irradiated at a distance of 92 cm. from the source, where the intensity was approximately 4.2 ergs per second per square millimeter. In a majority of the experiments, 0.05 milliliter of broth containing bacteria was spread over a surface about 8 cm. in diameter in a flat Petri dish 10 cm. in diameter, so that the walls of the dish would not shade the bacteria. After exposure, the material was washed out with a known quantity of broth and used in the experiments.

X-ray treatment was done at the Memorial Hospital in New York City, under the supervision of Miss E. Focht, through the courtesy of Mr. L. D. Marinelli. A bacterial suspension in broth was irradiated in small glass tubes, with unfiltered rays generated at 180 kv. and 25 Ma. at an intensity of 2050 roentgens per minute.

Experimental Results.—Experiments with ultraviolet: In the first set of

experiments, we used a 24-hour-old culture of bacteria, which was concentrated by centrifuging so as to contain about 10^9 bacteria per milliliter. Samples of this suspension were irradiated in thin layers, then resuspended in suitable amounts of broth to give comparable concentrations of living bacteria in all samples.

The numbers of living bacteria in each sample were determined by plating and colony counts. The original suspension was also tested for number of *B/1* mutants. When exceptionally high numbers of mutants were present in the original culture, they would mask any increase in mutation rate produced by the radiation; and therefore experiments in which this happened were not taken into account.

From each of the irradiated and control samples, twelve portions of 0.1 ml. were plated on different plates. Three plates from each sample were phage treated immediately, three were treated after 2 hours of incubation, three after 3 hours, and the last three after 4 hours. These plates were then incubated, and counts of phage-resistant colonies were made after 48 hours.

TABLE 1

SUMMARY OF 7 EXPERIMENTS, SHOWING THE NUMBER OF *B/1* MUTANTS AMONG *B* BACTERIA TREATED WITH VARIOUS DOSAGES OF ULTRAVIOLET AND INCUBATED FOR VARIOUS LENGTHS OF TIME BEFORE APPLICATION OF PHAGE

INCUBATED BEFORE PHAGING	NUMBER OF *B/1* COLONIES AFTER IRRADIATION OF 4 MIN.	2 MIN.	1 MIN.	CONTROL
0	19	6	4	0
2 hrs.	118	95	24	4
3 hrs.	548	414	226	55
4 hrs.	2081	1234	486	295
No. of bacteria plated	1.12×10^7	1.23×10^7	8.61×10^6	2.42×10^7

To determine the growth rate of bacteria after irradiation, dilutions from each sample were also incubated in broth and plated at intervals. From these platings, the number of bacteria present on the plates at various times was calculated, on the assumption of similar rates of growth on agar and in broth.

With minor variations, the procedure outlined above was used in the seven experiments summarized in table 1. This table shows the total number of bacteria plated in each series and the numbers of *B/1* colonies found on Petri dishes where the phage had been added immediately after irradiation or after incubation periods of 2, 3 or 4 hours. It is evident that in all tests made with treated series the numbers of *B/1* colonies were considerably higher than in the control series.

The larger number of *B/1* in the series where the phage was applied immediately after irradiation indicates that the ultraviolet treatment was effective in inducing *B/1* mutants which appeared before the first bacterial division had been completed.

TABLE 2

ANALYSIS OF DATA FROM TABLE 1, GIVING ESTIMATES OF THE MUTATION RATES

INCUBATED BEFORE PHAGING	4 MIN.			2 MIN.			1 MIN.			CONTROL		
	NO. OF BACTERIA	B/1	RATE × 10⁸	NO. OF BACTERIA	B/1	RATE × 10⁸	NO. OF BACTERIA	B/1	RATE × 10⁸	NO. OF BACTERIA	B/1	RATE × 10⁸
0	1.12×10^7	19	170	1.23×10^7	6	49	8.61×10^6	4	46	2.42×10^7	0	...
2 hrs.	1.79×10^7	118	...	2.71×10^7	95	...	3.79×10^7	4	..	2.4×10^8	4	...
Increment 0–2 hrs.	0.67×10^7	99	1478	1.48×10^7	89	601	2.93×10^7	20	68	2.2×10^8	4	1.8
3 hrs.	1.43×10^8	548	...	2.17×10^8	414	...	3.03×10^8	226	..	19.2×10^8	55	...
Increment 2–3 hrs.	1.25×10^8	430	344	1.90×10^8	319	168	2.65×10^8	202	76	16.8×10^8	51	3.0
4 hrs.	11.44×10^8	2081	...	17.36×10^8	1234	...	24.24×10^8	486	..	153.6×10^8	295	...
Increment 3–4 hrs.	10.01×10^8	1533	153	15.19×10^8	820	54	21.21×10^8	260	12	134.4×10^8	240	1.8

Growth tests made in broth showed that the initial period of lag in the growth (lag period) is lengthened in treated bacteria, an observation which has already been made by Hollaender and Duggar.[4] After an incubation period of 2 hours, the number of bacteria in the control series increased on the average by a factor of 10, the number in material treated for one minute increased 4.4 times, that in material treated 2 minutes 2.2 times, and that in material treated 4 minutes 1.6 times. After the lag period had passed, both treated and control bacteria multiplied regularly, doubling their number once in about every 20 minutes. No study was made of the multiplication of bacteria on agar, but it is unlikely that there is any considerable difference between the rates of growth on agar and in broth during the several early divisions before the number is large enough to produce crowding on agar. Indeed, observations made under the microscope indicate that the length of the lag phase on agar is about the same as that in broth.

Thus when the phage was applied to cultures that had been incubated for 2 hours in the series that received 4-minute irradiation the bacteria were just passing through the first division, while in the other three series they had already passed through one, two, and more than three divisions, respectively, by the time the phage was applied. Table 1 shows that the number of *B/1* mutants, where plates were incubated for 2 hours before adding phage, was very much greater than in the controls. A similar situation obtained in the case of cultures that were incubated for 3 and for 4 hours before phage treatment. It is evident that new *B/1* mutants continued to appear at a higher

rate in the treated series than in the controls, even after the bacteria had passed through several divisions.

From the data given in table 1, and from the calculated number of bacteria present at each time, the rate of mutation of *B* to *B/1* may be estimated. Such an analysis of the data is presented in table 2. For the cultures where phage was applied immediately after treatment, the mutation rate was calculated directly from the number of bacteria plated and the number of *B/1* colonies observed. In order to calculate the mutation rate among bacteria that were formed by division of plated individuals during the 2 hours of incubation, the total number of bacteria present after that time had to be estimated. This was done by multiplying the number of plated bacteria by 1.6, 2.2, 4.4 and 10 for the 4-, 2- and 1-minute series and the controls, respectively. As has already been pointed out, these values represent average increases in the number of bacteria and were determined by parallel experiments made in broth.

Since the rate of division of irradiated bacteria becomes normal once they pass the lag phase, and since the lag phase is completed in all series after 2 hours of incubation, it is assumed that from then on the number of individuals in each of the series doubles every 20 minutes. For each one-hour period, then, the number increases by a factor of 8. Consequently, values for the total number of bacteria present after 3 and 4 hours of incubation were obtained by multiplying by 8 the estimated values at the end of the previous hour.

It is realized that these estimates are only approximate. However, it is felt that the approximation is sufficiently close for the purpose at hand.

Examination of table 2 reveals that the calculated rate of mutation of *B* to *B/1*, per number of bacteria, increases in the treated series until the bacteria have passed through one or two divisions. Thereafter the rate begins to fall.

In order to determine if or when the rates reach the normal level of about 1×10^{-8}, a set of six experiments was performed in which bacteria were irradiated for 4 minutes and phage applied either immediately after the treatment or after incubation of 4, 5 or 6 hours. A summary of these experiments is presented in table 3, in the same form as the data shown in table 2. To obtain the estimate of the total number of bacteria present after 4 hours of incubation, the number of plated bacteria was multiplied by 102.4, which is equal to the $1.6 \times 8 \times 8$ used in table 2. Values for 5 and 6 hours were obtained by multiplying by 8 for each 1-hour period. It is evident from table 3 that the mutation rate continued to decrease with subsequent bacterial division and that at 6 hours, when the bacteria had passed through approximately 13 divisions, it had reached normal level.

Experiments with x-rays: A 48-hour culture of bacteria in broth was used as the source of material. About 1.3 cc. of bacterial suspension was

placed in each of three small glass tubes, which were then irradiated with 10,000, 20,000 and 50,000 roentgens, respectively. After irradiation, 15 platings of 0.05 cc. of the bacterial suspension were made on Petri dishes from each of the three tubes. Three Petri dishes from each set were exposed to phage aerosol immediately after plating, and others were exposed in groups of three after incubation of 1, 2, 3 and 4 hours. A summary of two experiments is given in table 4. The data are arranged as in table 2.

Tests made in broth indicated that the lag period of the bacteria was not materially affected by x-ray treatment. Under the conditions of these experiments, the number of bacteria doubled after 1 hour in broth at 37°C.

It is evident from table 4 that, as in the ultraviolet experiments, only a fraction of the induced mutants showed up immediately after treatment, while the appearance of a large proportion of them was delayed until after the treated bacteria had passed through several divisions. Comparisons of the data of tables 2 and 4 indicate obvious differences between the ultraviolet and the x-ray results. These are now being investigated further, and an analysis of this work will be presented in another paper.

TABLE 3

SUMMARY OF 2 EXPERIMENTS, WITH ESTIMATES OF THE RATES OF MUTATION OF *B* TO *B/1* AFTER ULTRAVIOLET TREATMENT OF 4 MINUTES FOLLOWED BY INCUBATION FOR VARIOUS LENTHS OF TIME BEFORE APPLICATION PHAGE

INCUBATED BEFORE PHAGING	NUMBER OF BACTERIA	*B/1*	RATE $\times 10^8$
0	2.25×10^8	7	311.1
4 hrs.	2.3×10^8	234	...
Increment 0–4 hrs.	.....	227	101.7
5 hrs.	18.4×10^8	297	...
Increment 4–5 hrs.	16.1×10^8	63	3.9
6 hrs.	147.2×10^8	543	...
Increment 5–6 hrs.	128.8×10^8	246	1.9

Discussion.—Changes induced by irradiations cannot be distinguished from those which occur spontaneously and which have already been extensively studied.[1, 2] In the course of these irradiation experiments several hundred *B/1* strains were grown in broth and tested for resistance to T1 and T2. They were found to be resistant to the first-mentioned phage and sensitive to the second, just as were the spontaneously originating strains described in previous experiments. Similar morphological types are found among both spontaneous and radiation-induced mutants—namely, a type that forms normal, large colonies and one that forms tiny colonies.

The simplest interpretation of the changes from *B* to *B/1* is that they are mutations comparable to gene mutations in higher organisms. However, since our knowledge of the mechanisms governing the transmission of heredity from parents to offspring in bacteria is still inadequate, other

TABLE 4

SUMMARY OF 2 EXPERIMENTS, WITH ESTIMATES OF THE RATES OF MUTATION OF *B* TO *B/1* AFTER X-RAY TREATMENT WITH VARIOUS DOSAGES AND INCUBATION FOR VARIOUS LENGTHS OF TIME BEFORE APPLICATION OF PHAGE

INCUBATED BEFORE PHAGING	10,000 r			20,000 r			50,000 r		
	NO. OF BACTERIA	*B/1*	RATE × 10^8	NO. OF BACTERIA	*B/1*	RATE × 10^8	NO. OF BACTERIA	*B/1*	RATE × 10^8
0	7.8×10^7	21	26.9	6.2×10^7	44	71.0	7.2×10^6	57	791.7
1 hr.	1.56×10^8	57	...	1.24×10^8	51	...	1.44×10^7	88	...
Increment 0–1 hr.	0.78×10^8	36	46.2	0.62×10^8	7	11.3	0.72×10^7	31	430.6
2 hrs.	12.5×10^8	148	...	9.9×10^8	224	...	1.2×10^8	291	...
Increment 1–2 hrs.	10.9×10^8	91	8.3	8.7×10^8	173	19.9	1.06×10^8	203	191.5
3 hrs.	100.0×10^8	318	...	79.2×10^8	353	...	9.6×10^8	378	...
Increment 2–3 hrs.	87.5×10^8	170	1.9	69.3×10^8	129	1.9	8.4×10^8	87	10.3
4 hrs.	.....	...	...	.....	...	...	76.8×10^8	569	...
Increment 3–4 hrs.	.....	...	...	.....	...	...	67.2×10^8	191	2.8

possibilities should be considered. Only by eliminating such alternative explanations can one hope to determine whether the hereditary mechanisms in bacteria are fundamentally similar to those in higher forms.

For example, the genes in bacteria may not be organized into chromosomes, and several or many genes of the same kind may be present in each individual. Recessive mutations—which, as is well known, constitute a large proportion of all mutations—would then attain phenotypic expression only after the individual had passed through several divisions, and the mutant gene had been sorted out and had replaced its allele in some individuals.

Certain other interpretations should be considered. All changes from *B* to *B/1* affect reactions between bacteria and phages. Since sensitive bacteria adsorb phages but resistant ones do not, and since adsorption is a surface phenomenon, it is possible that changes in the surface bring about a condition which ultimately may be responsible for the origin of resistance. It might be supposed that resistance originates as a change in a small sector of bacterial surface, for example, a receptor, and that it spreads in successive divisions so that it finally affects one whole bacterium, which then reproduces as a resistant mutant.

However, the evidence presented here shows that some of the induced mutations become phenotypically effective at the time of the first bacterial division. This would indicate that the mechanism responsible for the transmission of resistance to phage is neither

one involving many genes of the same kind, as first suggested, nor a surface phenomenon which would require several cell divisions before being manifested.

Two points should be particularly emphasized in discussing the data presented in this paper: (1) that some of the induced mutants are phenotypically effective by the time the first bacterial division occurs, and (2) that new mutants continue to appear during several bacterial generations.

It is known from the work of Delbrück[5] that one sensitive bacterium may adsorb as many as 250 phages, while resistant bacteria do not adsorb any. Presumably an adsorbed phage enters a bacterium and multiplies therein until finally the bacterium is lysed—it bursts and liberates phage particles Therefore, there are two critical steps in the life of a bacterium which determine whether or not it will be lysed by phage if this is present. These steps are the adsorption of phage by the surface and the multiplication of phage within a bacterium. It is evident that if either of these two processes is prevented from occurring a bacterium will become resistant to phage, and that if a mutation in a bacterium is capable of making it resistant it must be capable of blocking one of these two processes. If the adsorption of phage were immediately blocked by a mutated gene, this would mean that such a gene exerted an immediate influence on the organism and affected the forces active in adsorption. On the other hand, if a mutation prevented the multiplication of phage, this might mean that the mutated gene became active during the bacterial division, since, as a rule, the multiplication of phage is intimately connected with the division of bacteria.

Experiments were designed in such a way as to leave very little doubt that *B/1* mutants that appeared after bacteria had passed through several divisions were due either to mutations that originated after irradiation or mutations that were induced at the time of irradiation but failed to manifest their effect until the organism had passed through several cell divisions.

The simplest way to explain the appearance of mutants immediately after treatment as well as after several bacterial divisions would be to assume that these bacteria are diploid, and that the immediate mutants are a result of coincidental changes of both alleles by two independent hits, while the delayed mutants appear as a result of segregation of heterozygotes. Two objections make this hypothesis improbable. One of them is the large number of immediate mutants found in the ultraviolet and, particularly, the x-ray experiments. The observed numbers of these mutants differed from the calculated values by a factor of about 10 in the ultraviolet experiments and by a factor of about 1000 in the x-ray experiments. These discrepancies could be accounted for by assuming that some of the homozygous mutants could be induced by a single hit—particularly in the case of x-rays. Such an assumption would not be unreasonable, since Lea and Catcheside[6] have estimated that the field of action of one x-ray

ionization is about 0.1 micron, so that any two alleles that happened to be located that close to each other would have a chance of being affected together.

The other, and more serious, objection against this hypothesis is the distribution of mutants. A few of them appear immediately after treatment, but the larger proportion do not express themselves until after several cell divisions have taken place. If delayed mutants were due either to a simple segregation of a heterozygote or to aberrant divisions of asexual cells resulting in segregations, it would be expected that the largest proportion of delayed mutants would appear during the second bacterial generation, and that the frequency would rapidly diminish thereafter.

Another possible explanation of the observed delay in the appearance of mutants assumes that the bacterial population used in experiments was not homogeneous—that is, that some bacteria were haploid, others diploid, and still others polyploid. By assuming certain proportions of the various types, a mixture could be postulated which would give the observed results. In all the experiments reported so far, resting bacteria were used; these were obtained by growing bacteria in broth, at 37°C., for either 24 or 48 hours. It is known that growing bacteria are physiologically very different from resting bacteria. Since the proportions of haploid, diploid and polyploid individuals—if these did occur at all—might be expected to be different in different physiological conditions, we compared data obtained from ultraviolet irradiation of resting and actively growing bacteria. The results were not significantly different.

Another possible hypothesis assumes that all mutations occur during irradiation but that some are delayed in their manifestation until the supply of the substrate manufactured by the gene and necessary for the production of material that makes the bacteria sensitive to the phage is exhausted. It might then be expected that changes in the environment might affect the rate of utilization of the substrate.

Still another hypothesis may be considered to explain the results obtained. It may be assumed that two types of change are induced by irradiation—one type producing gene changes, and the other inducing some change in the cell, either of the chromosomes or of the cytoplasm, which increases the mutability of the gene system. The latter type of change would decrease in effectiveness with each cell division, and after a number of divisions lose its potency. According to this hypothesis, the calculated mutation rate per number of bacteria would be highest after the early divisions, a situation observed in our experiments (table 2).

In irradiation work with Drosophila a similar delayed effect is observed after treatment of sperm. In that case mosaics, or "fractional" mutants, are obtained. Muller[7] and several other workers have interpreted this observation by assuming that the chromosomes in some of the sperms are

already split, and that in such cases the irradiation produces a change in only one of the two strands. Neuhaus,[8] however, after studying the distribution of the size of mosaic regions, reached the conclusion that these mosaics are due to new mutations occurring as a result of a delayed effect of the irradiation.

In the experiments described in this paper various doses of ultraviolet radiation and x-rays were used. The data indicate an increase in the effect with increase of the dosage. Specially designed experiments are now under way, however, to determine the relationship between the dosage and the number of induced mutants; this problem will be discussed elsewhere. An effort is being made also to devise experiments which will differentiate between the various possibilities for interpretation of the results of experiments discussed here.

Summary.—The rate of mutation from sensitivity to resistance to bacteriophage T1 in the *B* strain of *E. coli* was measured after treatment with ultraviolet radiation (2537 A) and with x-rays.

Both radiations produced a substantial increase in the mutation rate, which increase became greater with higher doses of radiation.

Some of the induced mutations manifest themselves by the time the treated bacteria begin to multiply, while the majority of them express themselves after the bacteria have passed through several divisions.

Several possibilities for interpretation of results are discussed.

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ON THE NUMBER OF SOLUTIONS OF SOME GENERAL TYPES OF EQUATIONS IN A FINITE FIELD

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Consider the equation

$$c_1x_1^{a_1} + c_2x_2^{a_2} + \ldots + c_sx_s^{a_s} + c_{s+1} = 0 \qquad (1)$$

in the x's, where the a's are integers such that $0 < a \leqq p^n - 1$; $s \geqq 2$; the c's belong to a finite field of order p^n, p prime, which will be designated by $F(p^n)$; and

$$c_1c_2 \ldots c_sx_1x_2 \ldots x_s \neq 0$$

in $F(p^n)$. The problem discussed here will be that of *determining directly* the number of solutions $x_1^{a_1}, x_2^{a_2}, \ldots, x_s^{a_s}$ in $F(p^n)$ of (1). By "determining directly" we shall mean the determination of this number in a finite number of steps without trial. When $n = 1$ it is possible to carry this out using a method given by the writer,[1] but this scheme leads to very complicated procedures when $s > 2$ in (1). In the present paper we shall employ other methods. The main result (Theorem I) established reduces the general problem to the determination of the number of solutions y_1^m and y_2^m in

$$cy_1^m + dy_2^m + 1 = 0; \qquad y_1y_2 \neq 0$$

for some integer m depending on the a's and p^n, with c and d any given non-zero elements of $F(p^n)$ *provided a table of indices for* $F(p^n)$ *is known*, that is, if g is a generator of the cyclic group formed by the non-zero elements of $F(p^n)$ then we know the value of z in

$$g^z = k; \qquad 0 \leqq z < p^n - 1$$

for a given k, and if z is given we know k. We write $z = \text{ind } k$.

Using indices we first consider (1) with $c_{s+1} \neq 0$. After dividing through by this element our equation may be put in the form

$$1 + E(e_1 + \alpha_1a_1) + E(e_2 + \alpha_2a_2) + \ldots + E(e_s + \alpha_sa_s) = 0 \quad (2)$$

with $0 \leqq e_i < a_i$; $i = 1, 2, \ldots, s$, and where $E(t)$ denotes g^t. In this relation it is sufficient to take the α's in the range $0, 1, 2, \ldots, (p^n - 1)/d_i - 1$, where $(a_i, p^n - 1) = d_i$; $i = 1, 2, \ldots, s$. Also there is a one-to-one correspondence[2] between a solution $E(\alpha_1a_1), E(\alpha_2a_2), \ldots, E(\alpha_sa_s)$ of (2) and a certain solution $E(\beta_1d_1), E(\beta_2d_2), \ldots, E(\beta_sd_s)$ of

$$1 + \sum_{i=1}^{s} E(e_i + \beta_i d_i) = 0 \qquad (3)$$

where β_i lies in the range

$$0, 1, 2, \ldots, (p^n - 1)/d_i - 1; \qquad i = 1, 2, \ldots, s.$$

By the use of the Euclidean algorithm in connection with the exponents we see that in the consideration of (3) it is sufficient to take each $e_i < d_i$.

For any value of β_1, say β, appearing in (3) we may write

$$\beta = w + \gamma \frac{m}{d_1}, \qquad 0 \leqq w < \frac{m}{d_1},$$

where m is the L.C.M. of the d's in (3). Then

$$e_1 + \beta d_1 = (e_1 + w d_1) + \gamma m$$

and

$$e_1 + wd = e_1 + d_1\left(\frac{m}{d_1} - 1\right) = e_1 - d_1 + m < m,$$

since $d_1 > e_1$. This β then gives a solution in $E(\gamma m)$, $E(\beta_2 d_2)$, ... $E(\beta_s d_s)$ of the equation

$$E(e_1 + wd_1 + \gamma m) + E(e_2 + \beta_2 d_2) + \ldots + E(e_s + \beta_s d_s) + 1 = 0,$$

with $0 \leqq \gamma < (p^n - 1)/m$.

Conversely any solution in $E(\delta m)$, $E(\beta_2 d_2)$, ..., $E(\beta_s d_s)$ of

$$E(e_1 + vd_1 + \delta m) + E(e_2 + \beta_2 d_2) + \ldots + 1 = 0$$

for a given v such that $0 \leqq v < \frac{m}{d_1}$ with $0 \leqq < \delta\ (p^n - 1)/m$, gives a solution of (3) for we have

$$e_1 + vd_1 + \delta m = e_1 + d_1\left(v + \delta \frac{m}{d_1}\right)$$

and it is found that $v + \delta \frac{m}{d_1}$ is in the range $0, 1, 2, \ldots, ((p^n - 1)/d_1 - 1)$.

Hence $v + \delta \frac{m}{d_1}$ is a value for β_1 in (3). In view of this we may obtain the number of solutions of (3) in

$$E(\beta_1 d_1), E(\beta_2 d_2), \ldots, E(\beta_s d_s)$$

by taking the sum of the numbers of solutions in

$$E(\gamma_1 m), E(\beta_2 d_2), \ldots . E(\beta_s d_s) \text{ of each of the equations}$$

$$E(k_1) + E(e_2 + \beta_2 d_2) + E(e_3 + \beta_3 d_3) + \ldots . E(e_s + \beta_s d_s) + 1 = 0 \quad (4a)$$

where $k_1 = e_1 + w_1 d_1 + \gamma_1 m$, and w_1 ranges over each element of the set $0, 1, 2, \ldots ., \left(\frac{m}{d_1} - 1\right)$, and γ_1 is to be determined so that $0 \leqq \gamma_1 < (p^n - 1)/m$. We now proceed with the exponent $e_2 + \beta_2 d_2$ in (4a) as we did with the exponent $e_1 + \beta_1 d_1$ in (3) and we obtain the result that the number of solutions of (3) in

$$E(\beta_1 a_1), E(\beta_2 a_2), \ldots ., E(\beta_s a_s)$$

s the sum of the number of solutions in

$$E(\gamma_1 m), E(\gamma_2 m), E(\beta_3 a_3), \ldots ., E(\beta_s a_s)$$

of each of the equations

$$E(k_1) + E(k_2) + E(e_3 + \beta_3 a_3) + \ldots . + E(e_s + \beta_s a_s) + 1 = 0; \quad k_2 = e_2 + w_2 d_2 + \gamma_2 m; \quad (4b)$$

where w_1 ranges as before in k_1 and w_2 ranges over the set $0, 1, 2, \ldots ., \left(\frac{m}{d_2} - 1\right)$. By proceeding in this manner with the other terms in (4b) we derive the result that the number of solutions of (3) equals

$$\sum_{w_1, w_2, \ldots, w_s} (e_1 + w_1 d_1, e_2 + w_2 d_2, \ldots ., e_s + w_s d_s) \quad (5)$$

where w_i ranges independently over the set $0, 1, 2, \ldots ., \left(\frac{m}{d_i} - 1\right)$; $i = 1, 2, \ldots ., s$, and the symbol

$$(f_1, f_2, \ldots ., f_s)$$

means the number of solutions in

$$E(\epsilon_1 m), E(\epsilon_2 m), \ldots ., E(\epsilon_s m)$$

of

$$E(f_1 + \epsilon_1 m) + E(f_2 + \epsilon_2 m) + \ldots . + E(f_s + \epsilon_s m) + 1 = 0.$$

We next proceed to show that the number of solutions of

$$1 + \sum_{i=1}^{s} E(u_i + m r_i) = 0 \quad (6)$$

for a given $u_1, u_2, \ldots ., u_s$ may be obtained directly if we know the number of solutions of an equation of the type

$$1 + E(v_1 + m t_1) + E(v_2 + m t_2) = 0 \quad (7)$$

for a given v_1 and v_2. For the necessary argument we shall find it con-

venient to introduce the notation θ_u for $E(u + mr)$ so that in place of (6) we have

$$\theta_{u_1} + \theta_{u_2} + \ldots . + \theta_{u_s} + 1 = 0. \tag{8}$$

In other words θ_{u_i} is any quantity in $F(p^n)$ whose index is congruent to u_i modulo m. Denote the number of solutions in $\theta_{u_1}, \theta_{u_2}, \ldots ., \theta_{u_s}$ of (6) by $(u_1, u_2, \ldots ., u_s)$. We first note that for a given h and t

$$\theta_{u_1} + \theta_{u_2} + \ldots . + \theta_{u_s} + E(h + tm) = 0 \tag{9}$$

reduces, when we divide by the last term on the left, to

$$\theta_{u_1 - h} + \theta_{u_2 - h} + \ldots . + \theta_{u_s - h} + 1 = 0. \tag{10}$$

Consider (8) for $s \geqq 3$. To enumerate its solutions we proceed by induction. We assume that $(u_1, u_2, u_3, \ldots ., u_{s-1})$ for any integers u may be determined directly. For $s = 3$, this reduces to the assumption that the number of solutions of (7) may be determined directly. We shall then prove the same thing for $(u_1, u_2, u_3, \ldots ., u_s)$. Now (8) may be written in the form

$$(\theta_{u_1} + \theta_{u_2} + \ldots . + \theta_{u_{s-2}} + 1 - \theta_i) + (\theta_i + \theta_{u_{s-1}} + \theta_{u_s}) = 0.$$

Corresponding to each solution of

$$\theta_{u_1} + \theta_{u_2} + \ldots . + \theta_{u_{s-2}} + 1 - \theta_i = 0 \tag{11}$$

there must be a solution of

$$\theta_i + \theta_{u_{s-1}} + \theta_{u_s} = 0$$

in order that (8) may be satisfied. Using (9) and (10) the number of solutions of the latter is $(u_{s-1} - i, u_s - i)$ for a fixed i. Hence the number of solutions of (8) is

$$\sum_{i=1}^{m-1} (i + \beta, u_1, u_2, \ldots ., u_{s-2})(u_{s-1} - i, u_s - i); \tag{12}$$

$\beta = ind\ (-1)$; except that in (11) $\theta_{u_1}, \theta_{u_2}, \ldots ., \theta_{u_{s-2}}$ might be such that

$$\theta_{u_1} + \theta_{u_2} + \ldots . + \theta_{u_{s-2}} + 1 = 0 \tag{13}$$

and since this is impossible in (9) with $\theta_i \neq 0$, then this case was not included in our enumeration. The relation (13) used in (8) gives

$$\theta_{u_{s-1}} + \theta_{u_s} = 0 \tag{14}$$

so that when this is satisfied we have in place of (12)

$$\sum_{i=0}^{m-1} (i + \beta, u_1, \ldots, u_{s-2})(u_{s-1} - i, u_s - i) + \frac{p^n - 1}{m} (u_1, u_2, \ldots, u_{s-2}), \quad (15)$$

since it may be shown by known methods that there are $(p^n - 1)/m$ solutions of (14). By our assumption each term in (12) as well as (15) may be determined directly so either (12) or (15) is fully determined. Hence our induction is complete.

To obtain (2) we assumed that $c_{s+1} \neq 0$. The case where $c_{s+1} = 0$ does not reduce immediately to the case where we have a term unity in our equation as in (2) when it happens that the a's are not all equal. Hence we now consider this second case. But if we proceed as we did with (2) we find that such an equation reduces to the solution of an equation of the type

$$E(b_1 + m\sigma_1) + E(b_2 + m\sigma_2) + \ldots + E(b_s + m\sigma_s) = 0$$

and this reduces to the form (6) when we divide by the last term. Hence we have the

THEOREM I. *Let g be a generator of the cylic group formed by the non-zero elements of a finite field of order p^n, p prime, and designated by $F(p^n)$. Further, let $a_1, a_2, \ldots, a_s$ be a set of positive integers; $(p^n - 1, a_i) = d_i$, $i = 1, 2, \ldots, s$; and assume that m is the L.C.M. of $d_1, d_2, \ldots, d_s$. Then the number of different solutions in $F(p^n)$*

$$E(\alpha_1 a_1), E(\alpha_2 a_2), \ldots, E(\alpha_s a_s); \; s > 1;$$

of the equation

$$1 + E(e_1 + \alpha_1 a_1) + E(e_2 + \alpha_2 a_2) + \ldots + E(e_s + \alpha_s a_s) = 0;$$

$e_i \geqq 0$; *or of the equation*

$$E(e_1 + \alpha_1 a_1) + E(e_2 + \alpha_2 a_2) + \ldots + E(e_s + \alpha_s a_s) = 0$$

may be determined directly if the number of solutions $E(\sigma_1 m)$, $E(\sigma_2 m)$ in $F(p^n)$ of

$$E(u_1 + \sigma_1 m) + E(u_2 + \sigma_2 m) + 1 = 0$$

is known for any integers u_1 and $u_2 \geqq 0$. Here $E(t) = g^t$. The determination may be carried out by the use of the formulae (5), (12) and (15).

COROLLARY I. *An equation of the type, with the c's given elements of $F(p^n)$,*

$$c_1 x_1^{a_1} + c_2 x_2^{a_2} + \ldots + c_s x_s^{a_s} + c_{s+1} = 0$$

may, by the use of indices, be reduced to one of the forms set out in Theorem I,

and thus the number of its different solutions, $x_1^{a_1}, x_2^{a_2}, \ldots, x_s^{a_s}$ *in* $F(p^n)$ *may be directly determined.*

When such numbers are found, known methods will readily yield the number of solutions $x_1, x_2, \ldots, x_s$ of the same equation.

In another paper[3] the writer determined the number of solutions in $F(p)$ of

$$1 + E(k_1 + 4\omega_1) + E(k_2 + 2\omega_2) = 0. \tag{16}$$

The method there employed may be also used to derive the number of solutions of various other trinomial congruences modulo p (which are isomorphic with equations in a finite field $F(p)$) when the given exponents are small, using the representation of p by means of various quadratic forms. An example is the equation obtained from (16) by putting 3 in the first exponent instead of 4. By employing Theorem I of the present paper together with some results due mainly to Dickson[4] it is possible to derive similar results. He obtained by the use of quadratic forms explicit expressions for the number of incongruent solutions $E(m\tau_1)$, $E(m\tau_2)$ of

$$E(l_1 + m\tau_1) + E(l_2 + m\tau_2) + 1 = 0 \tag{17}$$

for $m = 1, 2, 3, 4, 5, 6, 8, 9, 10, 12$. By using Theorem I of the present paper and Dickson's formulae just referred to, we obtain the number of solutions of (16) and more generally the number of solutions of (2) whenever $n = 1$ and the L.C.M. of $d_1, d_2, \ldots, d_s$ in (3) has one of the values given for m which are listed just below relation (17).

Mitchell[5] obtained arithmetical formulae which give explicitly the number of solutions of (17) in $F(p^n)$, n even, where an integer n_1 exists such that $p^{n_1} \equiv -1 \pmod{m}$. Using these results we may obtain formulae for the number of solutions of (2) whenever the L.C.M. of $d_1, d_2, \ldots, d_s$ is m, with p, n and m related as just stated. These equations may also be applied to (1) without the use of indices.

[1] These PROCEEDINGS, **30**, 362–367(1944).

[3] Dickson, *Amer. Jour. Math.*, **57**, 464(1935) proved this for the case $n = 1$ and all the a's are equal in (2), and our statement may be proved in the same way.

[3] These PROCEEDINGS, **31**, 173–175(1945).

[4] *Amer. Jour. Math.*, **57**, 391–424(1935); *Trans. Amer. Math. Soc.*, **38**, 188–194 (1935).

[5] *Ann. Math. II*, **18**, 120(1917).

PRIME NUMBER OF CONJUGATE OPERATORS IN A GROUP

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Suppose that every set of conjugate non-invariant operators of the group G involves the same prime number p of operators. The object of the present article is to determine some of the fundamental properties of the category of groups which satisfy this simple condition but are not otherwise restricted. The group of inner isomorphisms of G can be represented as an intransitive permutation group such that different letters are assigned to the different non-invariant operators of the group G. Each of the transitive constituents of this permutation group is of degree p since it is assumed that each of the non-invariant operators of G has exactly p conjugates under G. We shall first prove that each of the transitive constituents of the group of inner isomorphisms of G is a cyclic group of order p and of degree p.

Since the number of non-invariant conjugate operators of G is p the corresponding permutations in the group of inner isomorphisms of G cannot have more than p conjugates in its group of inner isomorphisms. Each of the transitive constituents of the group of inner isomorphisms of G must therefore be a transitive group of degree p which is either abelian or contains exactly p conjugates. As it must involve permutations of order p and none of these could have p conjugates since the number of the conjugates of an operator of order p in a transitive group of degrees p is always prime to p, it has been proved that all of the transitive constituents of the group of inner isomorphisms of G are regular groups of order p.

We have now established the following theorem: *If every non-invariant operator of a group has exactly p conjugates under the group its group of inner isomorphisms may be represented as an abelian intransitive permutation group all of whose transitive constituents are regular groups of order p. This group is of order p^m and of type l^m.* We can prove that m is always an even number in this theorem. It should be emphasized that the number of distinct operators in a set of conjugate operators of a group cannot be less than the number of operators in a set of conjugates of the corresponding operators in the group of inner isomorphisms. If the former number is greater than the latter it is a multiple of it. We shall now prove that m in the preceding italicized theorem is always even.

To prove this fact we may start with a subgroup of G which includes the central of G and whose order is p times the order of this central. This subgroup is abelian since the pth power of every operator of G appears in the central of G. It can be extended by an operator of G so as to obtain a sub-

group of G whose order is p^2 times the order of the central of G and whose central is the central of G. It should be emphasized that this subgroup transforms its own operators in exactly the same manner as these operators are transformed under G. Hence it results that whenever the order of G exceeds p^2 times the order of the central of G then the remaining operators of G must include operators which are commutative with every operator of this subgroup and have their pth power in the central of G. This results from the known theorem that if the operators of an invariant proper subgroup of a group are transformed under this subgroup in the same way as they are transformed under the group then the remaining operators of the group include at least one operator which is commutative with every operator of this invariant subgroup.

It results from the above that whenever the order of G exceeds p^2 times the order of its central then G contains a subgroup whose order is p^4 times the order of its central and that this invariant subgroup transforms its operators in exactly the same way as they are transformed under G if the order of G exceeds p^4 times the order of the central of G. Since this process may be continued until G is reached whenever m is even and only then, it has been proved that m is an even number in the italicized theorem noted above. The proof is largely based on the fact that the invariant subgroups whose orders are even powers of p times the order of the central of G have this central for their own centrals and transform their own operators in exactly the same way as these operators are transformed under G itself.

The order of a commutator of G can clearly not exceed p. To prove that the order of the commutator subgroup of G can also not exceed p it may be noted that if the order of the commutator subgroup would be divisible by p^2 we might suppose that t_1 and t_2 are two independent generators of the commutator subgroup of G. It may be assumed without loss of generality that the subgroup of G noted above whose order is p^2 times the order of the central of G and which includes this central has the group generated by t_1 as its commutator subgroup and includes s_1 and s_2 as its non-invariant operators which give rise to the commutator t_1. It may also be assumed that s_3 and s_4 are two other operators of G which are commutative with s_1 and s_2 but give rise to the commutator t_2. It would then follow that the product s_2s_3 would have more than p conjugates under G. As this is impossible it has been proved that *the commutator subgroup of G is of order p.*

It results from Sylow's theorem that G contains only one Sylow subgroup whose order is a power of p since each of its operators is supposed to have no more than p conjugates under G. Since the largest subgroup of G whose order is prime to p appears in the central of G it is also invariant under G. It therefore results that G contains two invariant subgroups which have only the identity in common and hence it involves the direct

product of these two subgroups. Since the order of this direct product is equal to the order of G it has been proved that *if a group has the property that each of its non-invariant operators has a given prime number of conjugates under the group then it is the direct product of the Sylow subgroup whose order is a power of this prime number and an abelian subgroup whose order is prime to the given prime number.* The former of these two subgroups may be the identity.

Since the direct product of G and any abelian group whatever is again a group in which every non-invariant operator has the property that it has exactly p conjugates under the group it results that the two factor groups of G which are such that one is abelian and every non-invariant operator of the other has exactly p conjugates under it can usually be selected in various different ways. The main interest in G is its Sylow subgroup whose order is a power of p. Hence we may restrict our attention for the present to the study of G when it is a group whose order is a power of p. When p is odd all the operators of such a group, besides the identity, may be of order p but when $p = 2$ some of the operators of such a group must be of order 4. The simplest example of such a group is then the ortic group and the simplest example of G when p is any odd prime number is the non-abelian group of order p^3 which involves no operator of order p^2.

In the octic group the number of the operators of order 2 is three more than the number of the operators of order 4. It may be of some interest to prove that this condition is also satisfied in the simplest group whose order is an arbitrary power of 2 and which satisfies the condition that each of its non-invariant operators has exactly two conjugates under the group. It was noted above that the order of such a group is an arbitrary odd power of 2 which is at least equal to the third power. This infinite system of simplest groups of this kind may be constructed by starting with the octic group and then constructing the direct product of this group and a group of order 2. This group of order 16 may then be extended by an operator of order 2 which is commutative with every operator of the given octic group and transforms each of the remaining operators into itself multiplied by the commutator of order 2 in the given octic group. We thus obtain the group of order 32 which belongs to the given infinite system and satisfies the condition that the number of its operators of order 2 is exactly three more than the number of its operators of order 4.

The system of odd prime power groups all of whose operators besides the identity are equal to this odd prime number includes one and only one abelian group for every power of this prime number but the number of such non-abelian groups for a given power of this prime number increases with the index of the power of this prime number. It follows from the preceding developments that this is always true even as regards the special class of these groups which satisfy the condition that each of the non-invariant

operators of the group has a number of conjugates under the group which is equal to this prime number p. The number of these groups of order p^m is $(m-1)/2$ when m is odd and $(m-2)/2$ when m is even since such a group is completely determined by the order of its central quotient group, and this order was proved above to be an even power of p whose index is less than m and greater than zero. In particular, there is one and only one such group of order p^3 as well as of order p^4 while there are two and only two such groups of each of the orders p^5 and p^6, etc.

From the preceding developments we can formulate the following theorem: *If a group has the property that each of its non-invariant operators has the same prime number of conjugates under the group then its central quotient group has for its order an even power of this prime number, is abelian, and involves only operators besides the identity whose common order is this prime number.* While the orders of the operators in the central quotient group of this category of groups are therefore completely determined by the given condition the orders of the operators of the group are not limited thereby. It is evident that all the groups of this category are solvable, and that the category exhibits the fundamental nature of the concept of sets of conjugate operators in a group. It may be noted that whenever the number of conjugates in each set of conjugate non-invariant operators of a group is a prime number it may be equal to the same prime number in each case.